i.p. or oral doses, however, remains to be established. Study of the regional distribution indicates that salbutamol markedly accumulates in regions outside the blood-brain barrier such as the pituitary and pineal glands. This might be relevant to possible sites of action of the drug which could influence these structures, thereby modifying the central events.

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REFERENCES

Borsini, F., Bendotti, C., Thurlby, P., Samanin, R. (1982) Life Sci. 30: 905–911

Francès, H., Puech, A. J., Chermat, , Simon, P. (1979) Pharmacol. Res. Commun. 11: 273–279

Glowinski, J., Iversen, L. L. (1966) J. Neurochem. 13: 655–669

Lecrubier, Y., Puech, A. J., Jouvent, R., Simon, P., Widlocher, D. (1980) Br. J. Psychiat. 136: 354–358

Lecrubier, Y., Jouvent, R., Puech, A. J., Simon, P., Widlocher, D. (1977) Nouv. Presse Med. 6: 2786

Lerer, B., Ebstein, R. P., Blemaker, R. H. (1981) Psychopharmacology 75: 169–172

Martin, L. E., Hobson, J. C., Page, J. A., C. Harrison (1971) Eur. J. Pharmacol. 14: 183–199

Martin, L. E., Rees, J., Tanner, R. J. N. (1976) Biomed. Mass Spectrom. 3: 184–190

Oosterhuis, B., Van Boxtel, C. J. (1982) J. Chromatogr. 232: 327–334

Ortmann, R., Martin, S., Radeke, E., Delini-Stula, A. (1981) Naunyn-Schmiedeberg's Arch. Pharmacol. 316: 225–230

Przegalinski, E., Baran, L., Kedrek, G. (1980) Pol. J. Pharmacol. Pharm. 32: 485–493

Sugrue, M. F. (1982) J. Pharm. Pharmacol. 34: 446–449

Waldmeier, P. C. (1981) Naunyn-Schmiedeberg's Arch. Pharmacol. 317: 115–119

Widlöcher, D., Lecrubier, Y., Jouvent, R., Puech, A. J., Simon, P. (1977) Lancet 2: 767–768

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Quinidine-induced rise in ajmaline plasma concentration

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A high-performance liquid chromatographic method is described for the simultaneous determination of ajmaline and quinidine in human plasma. With 0·5 ml plasma samples of ajmaline and quinidine, concentrations as low as 0·001 and 0·01 μg ml^{-1}, respectively, could be detected and the technique could be used to investigate the effect of quinidine on the pharmacokinetics of ajmaline. Four healthy subjects were given oral ajmaline (50 mg) alone or in combination with quinidine sulphate (200 mg) on separate occasions. When ajmaline was administered alone, its plasma concentrations were less than 0·03 μg ml^{-1}. Quinidine induced a marked increase to give a mean peak concentration of ajmaline which increased from 0·018 μg ml^{-1} after a single administration to 0·141 μg ml^{-1} in combination with quinidine. The area under the ajmaline concentration-time curves was increased 10 to 30-fold by the concurrent administration of quinidine. According to the one compartment open model, the absorption rate constant of ajmaline did not change appreciably, but the elimination rate constant was reduced to approximately 50% of the value in the absence of quinidine. The results indicate the existence of a significant interaction between oral ajmaline and quinidine.

The routine determination of ajmaline is hampered by the low sensitivity, time consumption and poor specificity of the available methods (Kleinsorge & Gaida 1961; Dombrowski et al 1975; Clemans et al 1977). Therefore, its pharmacokinetic properties have not been well understood (Kleinsorge & Gaida 1962: Dombrowski et al 1975; Spilker et al 1975; Iven 1977; Anttila et al 1978) and nothing is known about its kinetic behaviour in the clinical situation, where it is often used in combination with agents such as quinidine.

A specific and sensitive high-performance liquid chromatographic (hplc) method for the determination of ajmaline permits measurement of both ajmaline and quinidine in plasma simultaneously. This technique has been used to investigate the effect of quinidine on the pharmacokinetics of ajmaline given orally to healthy volunteers.

Materials and methods

Ajmaline was supplied by Sigma (St Louis, USA), quinidine was obtained from Nakarai Chem. Ltd (Kyoto, Japan) and quinidine ethylcarbonate, used as the internal standard, was purchased from Hoei Yakko (Osaka, Japan). Standard solutions of ajmaline and quinidine (1·00 mg ml^{-1}, respectively) were prepared weekly in 2% acetic acid and stored at 4 °C. The internal standard solution of quinine ethylcarbonate in diethyl ether was prepared daily and protected from light.

Acetonitrile (Kantokagaku, Tokyo, Japan) and glacial acetic acid (Wako, Osaka, Japan) was used without further purification. All other chemicals were analytical grade products available commercially.

A Shimadzu Model LC-3A high performance liquid chromatograph equipped with a Shimadzu RF-500LC fluorescence spectromonitor and a Zorbax CN column

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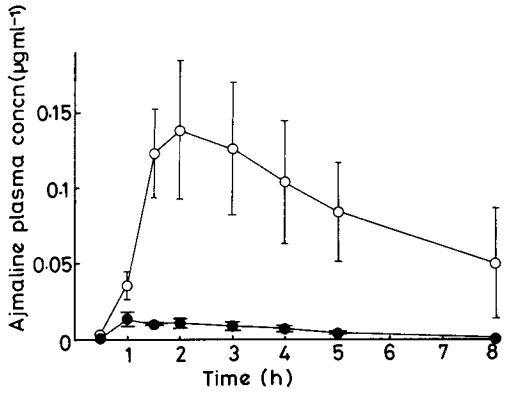

FIG. 1. Plasma concentrations of ajmaline after oral administration with or without quinidine in normal male volunteers. Each point and vertical bar represents the mean and standard error of four subjects. ● Ajmaline 50 mg alone; ○ ajmaline 50 mg with quinidine sulphate 200 mg.

(25 cm × 4·6 mm i.d.; 5 μm particle size; Shimadzu-Dupont, Kyoto, Japan) was used. The mobile phase was acetonitrile–2·5% acetic acid (44:56, v/v). A flow rate of 2·5 ml min^{-1} was used yielding an operating pressure of 170 kg cm^{-2}. The fluorescence spectromonitor had a 12 μl cell volume and was operated at an excitation wavelength of 295 nm and an emission wavelength of 375 nm.

Analytical procedure

To 0·5 ml of heparinized plasma in a 12-ml screw-capped glass tube were added 1·0 ml of glycine buffer (pH 10, 0·1 M) and 5·0 ml of diethyl ether containing 0·3 μg ml^{-1} of quinine ethylcarbonate as the internal standard. Each tube was vortexed for 2 min and centrifuged for 10 min at 2000*g* and the upper organic phase (4 ml) was transferred to tapered centrifuge tubes. After addition of 0·1 ml of 2-propanol, the organic phase was evaporated under a stream of nitrogen. The residue was reconstituted in 0·5 ml of 2% acetic acid and a 50 μl aliquot was injected into the high-performance liquid chromatograph.

Standard curves were prepared by spiking blank human plasma with ajmaline and quinidine. Both compounds were included in a single solution of spiked plasma at the concentrations of 0·01, 0·02, 0·05, 0·1, 0·2 and 0·5 μg ml^{-1} for ajmaline and 0·1, 0·2, 0·5, 1·0, 2·0 and 5·0 μg ml^{-1} for quinidine, and assayed concurrently. The peak height ratios of ajmaline and quinidine to quinine ethylcarbonate were plotted against the concentration of ajmaline and quinidine, respectively to provide standard curves.

Protocol design for normal volunteers

Four healthy male volunteers, aged 28 to 37 years, weight 51 to 67 kg, participated. Each ingested a single dose (50 mg, one tablet) of ajmaline (Gilurytmal, Nippon Chemiphar, Tokyo) with 100 ml of water on two occasions. The first trial served as a 'control', without concurrent drug administration. At least 7 days later, for the second trial, 100 mg quinidine sulphate (powder, JP, Hoei Yakko, Osaka) was given orally in the evening and on the next morning 200 mg of quinidine sulphate was given with ajmaline. Subjects fasted from 9 pm the evening before and no food or fluid was allowed until 4 h after the dose of ajmaline. Blood samples were collected at 0, 0·5, 1, 1·5, 2, 3, 4, 5, 8 h after each dose of ajmaline. No adverse effects were experienced.

Table 1. Pharmacokinetic parameters of ajmaline (Aj) in normal volunteers following oral administration with or without quinidine (Q).*

	K_a (h^{-1})[b]		K_e (h^{-1})[b]		AUC (μg hr ml^{-1})[b]		AUC ratio
Subject	Aj	Aj + Q	Aj	Aj + Q	Aj	Aj + Q	(Aj + Q)/Aj
K.Y.	2·41	3·00	0·299	0·270	0·034	0·485	14·3
M.Y.	6·77	4·61	0·602	0·239	0·035	0·383	10·9
K.O.	4·56	2·12	0·379	0·223	0·038	0·707	18·6
A.M.	1·12	2·29	0·326	0·107	0·091	2·825	31·0
Mean	3·72	3·01	0·402	0·210	0·050	1·100	18·7
± s.e.	1·24	0·57	0·069	0·036	0·014	0·579	4·4

* Parameters were calculated from the one compartment open model by a non-linear iterative least squares method using a digital computer.
[b] K_a, the absorption rate constant; K_e, the elimination rate constant; AUC, the area under the plasma concentration time curve.

Data analysis

Pharmacokinetic parameters in terms of one compartment open model for individual subjects were determined by non-linear least squares regression (Nakagawa et al 1978) using a FACOM M-200 digital computer. Statistical analysis was using Student's *t*-test with $P = 0·05$ as the minimal level of significance.

Results and discussion

Analytical method

The extraction of ajmaline and quinidine from plasma over the pH range 8·0 to 12·5 was maximal for both drugs using diethyl ether at pH 10·0. Recoveries were ajmaline (90%) and quinidine (95%). Other solvents (isoamylalcohol, chloroform, benzene, n-hexane) were less efficient. The extraction efficiency of ajmaline was not affected by the addition of quinidine.

Both ajmaline and quinidine exhibit natural fluorescence, allowing their separation by liquid chromatography and detection with a fluorescence detector. The excitation and emission wavelengths used were optimal for ajmaline but still allowed satisfactory measurement of quinidine.

On chromatography of human plasma spiked with ajmaline and quinidine (0·1 and 2·0 μg ml^{-1}, respectively), the compounds eluted from the reversed-phase column with retention times of 5·2, 14·5 and 16·8 min for ajmaline, quinine ethylcarbonate and quinidine, respectively. No interference in blank plasma was observed at the retention times of the three compounds. The detection limit for ajmaline was 0·001 and for

quinidine, 0·01 μg ml^{-1}. At these concentrations, peaks three times the baseline noise were obtained.

Calibration curves were constructed by spiking blank human plasma with ajmaline 0·01–0·5 μg ml^{-1} and quinidine 0·1–5·0 μg ml^{-1}. Both drugs exhibited excellent linear calibration curves with intercepts through the origin and correlation coefficients >0·9998.

Propranolol, disopyramide, procainamide, lidocaine and verapamil did not interfere in the assay.

The reproducibility of the method was good, the coefficient of variation for both drugs being less than 5%.

Ajmaline–quinidine interaction in normal volunteers

The pharmacokinetics of ajmaline, administered alone and in combination with quinidine, were evaluated in four normal volunteers. Plasma concentrations of ajmaline following oral administration are shown in Fig. 1. When administered alone, ajmaline gave plasma concentrations less than 0·03 μg ml^{-1}, but with quinidine given concurrently, the maximal concentration increased from 0·018 ± 0·004 to 0·141 ± 0·044 μg ml^{-1} (mean ± s.e.m., $P < 0·05$). The pharmacokinetic parameters obtained in terms of the one compartment open model are shown in Table 1. The absorption rate constant (K_a) of ajmaline appeared to be not affected by quinidine, while the elimination rate constant (K_e) was reduced significantly ($P < 0·05$). Furthermore, the area under the plasma concentration time curve (AUC) was increased 10 to 30-fold by the concurrent administration of quinidine.

The peak plasma concentration of quinidine (1·34 ± 0·29 μg ml^{-1}) was reached in 0·5 to 2 h after the dose and the K_e of quinidine was 0·129 ± 0·015 h^{-1} in the presence of ajmaline. Guentert et al (1979) reporting the pharmacokinetics of quinidine in ten healthy male volunteers, found that the time to peak concentration varied from 23 to 121 min and the peak concentration ranged from 0·9 to 1·8 μg ml^{-1} at the oral dose of 3·74 mg kg^{-1} quinidine base. The mean K_e of quinidine was reported to be 0·103 h^{-1}. Our results were similar, which suggest the pharmacokinetics of quinidine may not be affected by the presence of ajmaline.

The basic observation we report here is that the plasma concentration after oral dosing with ajmaline is increased by oral quinidine. The elimination half life of ajmaline was increased about two-fold by quinidine. However, it is not enough to explain the 10 to 30-fold increase in AUC of ajmaline. The product FD/V of AUC × K_e, where F is the availability of the drug, D is the dose and V is the apparent volume of distribution (Wagner & Nelson 1964) increased from 0·019 ± 0·004 μg ml^{-1} with the single administration to 0·171 ± 0·046 μg ml^{-1} with quinidine ($P < 0·025$). The results suggest the increased availability of ajmaline by the coadministration of quinidine, provided that quinidine does not affect the distribution volume of ajmaline. Our data in normal subjects do not allow any conclusion on the mechanisms of this drug interaction.

The antiarrhythmic effect of ajmaline has been found to be satisfactory only after parenteral administration (Mertens et al 1973). We found plasma concentrations after ajmaline alone by mouth were less than 0·03 μg ml^{-1}, while plasma concentrations of ajmaline sufficient for antiarrhythmic therapy were obtained when 50 mg of ajmaline was administered orally with 200 mg of quinidine. The combination would appear to have potential in the treatment of premature ventricular contractions because of the increased plasma concentration of ajmaline (Sakurai et al 1982).

REFERENCES

Anttila, M., Tikkanen, R., Nieminen, L. (1978) Arzneim.-Forsch./Drug Res. 28: 397–399

Clemans, S. D., Davison, C., Koss, R. F., Dorrbecker, B., O'Melia, P. E., Ross, Jr., R. W., Edelson, J. (1977) Ibid. 27: 1128–1130

Dombrowski, L. J., Crain, A. V. R., Browning, R. S., Pratt, E. L. (1975) J. Pharm. Sci. 64: 643–645

Guentert, T. W., Holford, N. H. G., Coates, P. E., Upton, R. A., Riegelman, S. (1979) J. Pharmacokinet. Biopharm. 7: 315–330

Iven, H. (1977) Naunyn-Schmiedeberg's Arch. Pharmacol. 298: 43–50

Kleinsorge, H., Gaida, P. (1961) Arzneim.Forsch./Drug Res. 11: 1100–1102

Kleinsorge, H., Gaida, P. (1962) Klin. Wschr. 40: 149–151

Mertens, V. H. M., Schelbert, H. R., Kreuzer, H., Spiller, P., Schmiel, F. K. (1973) Arzneim.-Forsch./Drug Res. 23: 642–645

Nakagawa, T., Oyanagi, Y., Togawa, H. (1978) 'SALS, a computer program for statistical analysis with least squares fitting', Library Program of the University of Tokyo Computer Centre, Tokyo, Japan.

Sakurai, T., Kawai, C., Hori, R. (1982) Jpn Circ. J. 46: 837

Spilker, B., Shargel, L., Koss, R. F., Minatoya, H. (1975) Arch. Int. Pharmacodyn. 216: 63–78

Wagner, J. G., Nelson, E. (1964) J. Pharm. Sci. 53: 1392–1403